

DOCUMENT-IDENTIFIER: US 20030166030 A1

TITLE: Methods to study and mechanisms of biofilm-related antibiotic resistance

Detail Description Paragraph:

[0129] The microtiter plate assay was modified to measure the increase in resistance developed by the wild type strain when growing in a biofilm. The wells of the microtiter dish are inoculated with bacteria and biofilms are allowed to form on the walls of the wells for 24 hrs in the absence of any shear force. After the biofilms had formed on the wells of the microtiter dish, the spent medium was replaced with the same media containing an antimicrobial agent. In this case, we used the aminoglycoside antibiotic tobramycin (Tb), an antibiotic that targets protein synthesis. Tb was selected because it is the primary antibiotic used to treat cystic fibrosis patients with chronic *P. aeruginosa* lung infections (Banerjee, D., and D. Stableforth, Drugs, 60(5):1053-64, 2000; Bonsignore, C. L., Pediatr Nurs. 24(3):258-9, 1998; Ratjen, F, Int J Antimicrob Agents 17(2):93-6, 2001). After exposing the biofilms to Tb for 24 hours, the antibiotic-containing medium is removed and replaced with fresh antibiotic-free medium. Any bacteria surviving in the biofilm outgrow and repopulate the planktonic phase of the wells. Viable cells were detected by plating on rich medium. Using this assay, we determined that the minimal bacteriocidal concentration (MBC) of Tb for the wt biofilm grown cells is 0.4 mg/ml. The MBC of planktonic cells was determined by adding the antibiotic to cells at the time they were inoculated into the microtiter dish, incubating the cells in the presence of antibiotic for 24 hrs, and assessing cell viability by plating on rich medium. Using this assay, the planktonic MBC was shown to be 0.008 mg/ml, a 50-fold decrease relative to the biofilm-grown bacteria.

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1: Mol Microbiol. 2002 May;44(4):903-15.

Links

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J Urol. 2003 Jul;170(1):335.

Structural basis of tropism of Escherichia coli to the bladder during urinary tract infection.

Hung CS, Bouckaert J, Hung D, Pinkner J, Widberg C, DeFusco A, Auguste CG, Strouse R, Langermann S, Waksman G, Hultgren SJ.

Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110, USA.

The first step in the colonization of the human urinary tract by pathogenic Escherichia coli is the mannose-sensitive binding of FimH, the adhesin present at the tip of type 1 pili, to the bladder epithelium. We elucidated crystallographically the interactions of FimH with D-mannose. The unique site binding pocket occupied by D-mannose was probed using site-directed mutagenesis. All but one of the mutants examined had greatly diminished mannose-binding activity and had also lost the ability to bind human bladder cells. The binding activity of the mono-saccharide D-mannose was delineated from this of mannotriose (Man(alpha 1-3)[Man(alpha 1-6)] Man) by generating mutants that abolished D-mannose binding but retained mannotriose binding activity. Our structure/function analysis demonstrated that the binding of the monosaccharide alpha-D-mannose is the primary bladder cell receptor for uropathogenic E. coli and that this event requires a highly conserved FimH binding pocket. The residues in the FimH mannose-binding pocket were sequenced and found to be invariant in over 200 uropathogenic strains of E. coli. Only enterohaemorrhagic E. coli (EHEC) possess a sequence variation within the mannose-binding pocket of FimH, suggesting a naturally occurring mechanism of attenuation in EHEC bacteria that would prevent them from being targeted to the urinary tract.

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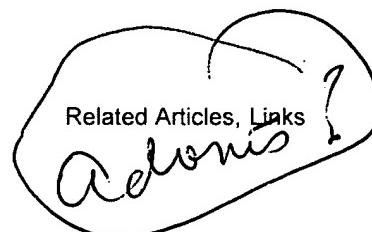
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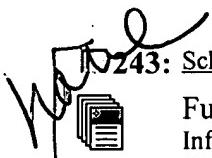
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